Cancer Chemother Pharmacol (1981) 5:193–196

Cancer Chemotherapy and Pharmacology

Deoxycoformycin: Neurological Toxicity

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Summary. *Deoxycoformycin (DCF) is a tight-binding inhibitor of adenosine deaminase (ADA) currently undergoing phase I–II evaluation. Neurological toxicity has been a frequent and occasionally severe complication of treatment with this drug. A T-cell leukemia patient with an Ommaya reservoir was treated with DCF, and the pharmacokinetics of the drug in the cerebrospinal fluid and plasma were studied. DCF penetrates the cerebrospinal fluid and achieves levels as high as $^1/_{10}$ the concurrent plasma levels. The accumulation of adenosine and deoxyadenosine in plasma, cerebrospinal fluid, and urine was monitored; the neuropharmacological effect of these metabolites is discussed.*

Introduction

Deoxycoformycin (DCF), a tight-binding inhibitor of adenosine deaminase (ADA; adenosine aminohydrolase, EC, 3.5.4.4) [1], is active in the treatment of acute lymphatic leukemia [3, 15]. The inhibition of ADA by DCF results in the accumulation of adenosine and deoxyadenosine [1]. Several hypotheses have been proposed to explain the toxic effects of adenosine or deoxyadenosine accumulation [17]. Recent evidence supports the accumulation of deoxyadenosine triphosphate (dATP) as the main mechanism for toxicity to lymphocytes [4, 6, 17, 19].

In the initial clinical trial [16], few complications of the drug were noted and no central nervous system (CNS) toxicity was reported. In our experience, lethargy has been a frequent complication of treatment with DCF [3]. Therefore, this study has been aimed at (1) determining the pharmacokinetics of DCF in the cerebrospinal fluid (CSF), and (2) attempting to correlate the degree of CNS depression with CSF levels of DCF or metabolites (adenosine or deoxyadenosine) expected to accumulate as a result of ADA inhibition by DCF. A patient with an Ommaya reservoir was treated on two occasions with DCF. This report describes the CSF pharmacokinetics of DCF in humans and monitors the accumulation of adenosine and deoxyadenosine in plasma, CSF, and urine.

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Materials and Methods

Description of Patient and Treatment Protocol. Patient P.G. is a 25-year-old male with T-cell leukemia. His initial therapy included vincristine, prednisone, and adriamycin combined with CNS prophylaxis. The patient subsequently relapsed with bone marrow and CNS disease. He received vincristine, prednisone, asparaginase, and intrathecal methotrexate via an Ommaya reservoir, with complete resolution of his disease. On his second relapse, DCF was given according to the following treatment protocol at 4-week intervals: 10 mg/m^2/day × 5 by IV bolus and 20 mg/m^2/day × 5 by IV bolus. On the third day of treatment at 10 mg/m^2, the patient became drowsy and increased his sleeping hours from 8–12; mental function was otherwise normal. On day 3 of treatment at 20 mg/m^2 the patient again became sleepy, and this progressed to somnolence by day 6 of treatment. This resolved over the next 3 days. Sequential samples of CSF and plasma were obtained for pharmacokinetic studies and CSF, plasma, and urine were monitored for accumulation of adenosine or deoxyadenosine.

Determination of DCF Concentrations in CSF and Plasma. The enzymatic assay used to determine levels of DCF in the CSF and plasma was a modification of previously described techniques [5, 9]. All plasma samples were heated at 100° C for 5 min to inactivate endogenous ADA activity and to free protein-bound DCF. Samples were then spun at 150,000 *g* for 90 min in a Beckman L5-65 ultracentrifuge with a Ti 40 rotor. Supernatant fluids were then filtered through Amicon CF-25 centriflo-membranes (Amicon Corp., Lexington, MA). The CSF samples were simply filtered through Amicon CF-25 centriflo-membranes (Amicon Corp., Lexington, MA). All samples were stored at −70° C prior to assaying for DCF levels.

A standard curve was constructed for the inhibition of ADA (calf intestinal ADA, 200 units/mg, Boehringer-Mannheim) by DCF. A reaction mixture containing 500 µl potassium phosphate

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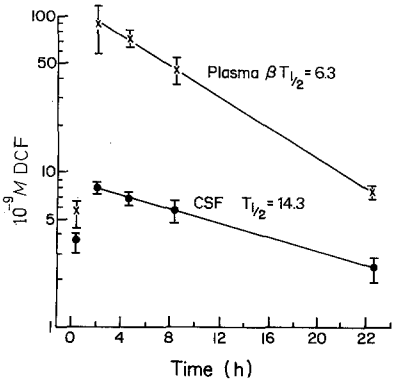

Fig. 1. Plasma and CSF concentrations of DCF (10 mg/m^2 × 5, day 3). The 95% confidence limits are indicated

buffer, 50 m*M*, pH 7.4; 100 µl DCF solutions (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 × 10^{-9} *M* DCF), and 200 µl of a solution of ADA (0.05 units of enzyme/ml) was incubated at 25° C for 5 min in a 1-ml cuvette. The clinical samples were assayed for DCF levels by adding 100 µl of the appropriate dilution of the prepared plasma or CSF samples instead of the DCF standard. After incubation, 200 µl 0.8 m*M* adenosine (Boehringer-Mannheim) was added and the reaction was followed at 265 nm in a recording Beckman model 25 spectrophotometer at 25° C. The ADA activity remaining after incubation with samples containing DCF is expressed as a percentage of the reaction rate of the control enzyme preparation containing no DCF. Appropriate controls were assayed concurrently with the clinical samples.

Determination of Adenosine and Deoxyadenosine Levels. The levels of adenosine and deoxyadenosine in the CSF, plasma and urine were determined by high-pressure liquid chromotography. The samples were prepared as described for measuring DCF levels and injected directly for analysis on an ALTEX HPLC machine equipped with a Hewlett-Packard integrator with an Ultrasphere ODS Altex column (details of method to be published). Adenosine and deoxyadenosine were identified by their retention time and confirmed by the peak-shift method after treatment with ADA.

Results

Levels of DCF in the Cerebrospinal Fluid and Plasma

Control plasma and CSF were free of ADA activity, and these samples did not inhibit the ADA added to the reaction assay when they were processed as indicated above. The recovery of DCF added to control plasma and CSF was greater than 90%. The dose-response curve was obtained by averaging the six determinations obtained at each drug concentration and using the method of least squares to obtain the following regression equation: concentration of DCF [nM] = −2.07 (± 0.02 SE) × log (fraction of control rate). The CSF and plasma levels of DCF represent the average of two determinations; 95% confidence limits on individual DCF levels were obtained with the aid of a previously described method [10] adapted for the no 'constant term' model. Figure 1 shows CSF and plasma DCF levels obtained on day 3, when the patient was receiving a dose of 10 mg/m^2; 95% confidence limits are indicated. CSF levels are approximately $^1/_{10}$ the plasma levels. The half-life of DCF in the CSF was 14.3 h, while the corresponding plasma excretion half-life was 6.3 h. The plasma half-lives on days 4 and 5 were 6.9 and 5.3 h, respectively. Plasma DCF levels were also measured each day 24 h after drug administration and there was an increase in the levels from 5.4 × 10^{-9} *M* on day 2 to 1.8 × 10^{-8} *M* on day 5. In contrast, there was no significant increase in CSF levels of DCF obtained concurrently. When the patient was receiving 20 mg/m^2, the CSF levels of DCF were also approximately $^1/_{10}$ the plasma levels. The half-lives were similar on both courses, and the plasma disappearance of DCF was exponential. For comparison, the plasma DCF levels determined by the intercept of the elimination curve with the ordinate on the concentration versus time plot were 1.7 × 10^{-7} *M* and 5.2 × 10^{-7} *M* on day 3 of courses at 10 mg/m^2 and 20 mg/m^2, respectively.

Adenosine and Deoxyadenosine Levels in the Cerebrospinal Fluid, Plasma, and Urine

Micromolar amounts of adenosine and deoxyadenosine accumulated in the plasma. Values as high as 4.2 and 9.1 µ*M* for adenosine and deoxyadenosine, respectively, were found when the patient received 20 mg/m^2 of DCF.

Deoxyadenosine levels increased in the urine during both courses of treatment with DCF, and reached values as high as 3 × 10^{-3} *M* on day 5 of the higher-dose treatment schedule (Fig. 2); no adenosine was detected in the urine during either course of treatment.

Adenosine was undetectable in the CSF during both courses of treatment, while deoxyadenosine levels of 1.3 µ*M* were identified only during the second course of treatment (20 mg/m^2).

Conclusion

DCF has shown activity in the treatment of acute lymphatic leukemia [3, 16]. Several clinical trials have been initiated as a result of these early studies [16]. CNS depression has been a frequent and sometimes severe complication of this treatment. The mechanism of this toxic effect remains unclear.

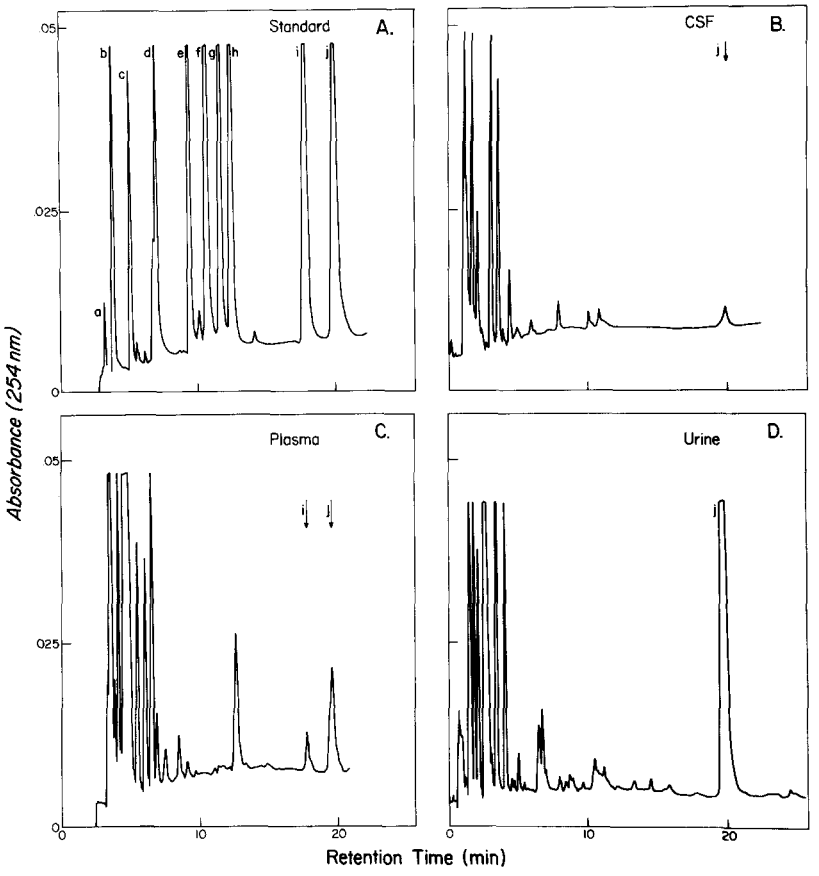

Fig. 2A–D. High-pressure liquid chromatography profiles of urine, plasma, and CSF (20 mg/m^2 × 5, day 5). **A** Standards: (a) uric acid, (b) hypoxanthine, (c) deoxycytidine, (d) adenine and deoxyuridine, (e) inosine, (f) deoxyinosine, (g) deoxyguanosine, (h) thymidine, (i) adenosine and (j) deoxyadenosine; **B** HPLC profile of CSF, showing accumulation of deoxyadenosine (1.3 μ*M*), but no adenosine; **C** HPLC profile of plasma, showing accumulation of adenosine (4.2 μ*M*) and deoxyadenosine (9.1 μ*M*); **D** HPLC profile of urine (24-h urine collection), showing accumulation of deoxyadenosine (3.0 m*M*)

DCF has been shown to penetrate the blood-brain barriers in animals at levels sufficient to inhibit ADA [9]. Peak CSF levels occur 2 or 3 h after injection of the drug in the dog and attain approximately $^1/_{10}$ the plasma levels; the excretion half-life is longer in CSF than in the plasma [5]. Our data shows that DCF crosses the human blood-brain barrier and also achieves CSF levels of approximately $^1/_{10}$ the concurrent plasma levels. However, in the clinical study, there was no delay in obtaining CSF and plasma peak levels as reported in dogs. This may simply represent species variation. It should be noted that the patient studied had received cranial radiation. In mice, brain radiation is known to increase the permeability of the blood-brain barrier [7], and a similar phenomenon may occur in humans and could explain the early CSF peak of DCF seen in our patient.

CNS depression increased when patient P.G. received DCF at 20 mg/m^2 × 5 compared to 10 mg/m^2 × 5. Concentrations of DCF in CSF varied according to dose with higher levels achieved at higher doses and similarly, higher levels of adenosine and/or deoxyadenosine were found in the plasma or CSF at higher dose levels.

The mechanism of CNS depression is unclear. EEG recordings have shown diffuse slowing of brain electrical activity in patients treated with this drug [3]. The intraventricular administration of adenosine to dogs causes profound CNS depression, as evidenced by increased drowsiness [8]. The intracerebral injection of DCF in rats potentiates the depressive effect of adenosine as measured by intracellular neuronal electrical activity [11]. In isolated rat brain tissue, DCF at concentrations of 5×10^{-10} *M* markedly potentiates the norepinephrine-induced accumulation of cyclic AMP, and this effect is significantly inhibited by theophylline, suggesting that DCF acts by causing an initial accumulation of adenosine [15]. Deoxyadenosine has also been shown to have a CNS-depressant effect on the firing of rat cerebral cortical neurons [12]. There is also good evidence for an adenosine receptor in the rat brain [13].

There is no information on the neuropharmacology of adenosine or deoxyadenosine in the human brain. However, in view of the experimental evidence obtained in animals, it appears most likely that the CNS depression observed in our patient was mediated by adenosine or deoxyadenosine. The human brain contains high levels of ADA [18], and

may be sensitive to the inhibition of this enzyme by DCF. CNS depression was more pronounced in our patient after administration of the higher dose of DCF (20 mg/m^2), when there was also greater accumulation of adenosine and deoxyadenosine.

Micromolar amounts of adenosine and deoxyadenosine were detectable in the plasma. It is possible that the CSF levels of adenosine, though too low for detection by our present methodology, achieve levels sufficient to produce CNS depression when ADA is inhibited. A blood-brain barrier for adenosine has been demonstrated in animals [2], CSF levels observed being approximately 10% of plasma levels. Our studies show that such a barrier exists for deoxyadenosine, and a similar barrier for adenosine seems very likely. In that event, it is likely that we would not have detected increases in CSF adenosine levels because plasma levels of adenosine were lower than those of deoxyadenosine, which were themselves at the lower limit of detectability in the CSF. Furthermore, intraparenchymal accumulation of adenosine secondary to ADA inhibition in the brain could mediate CNS depression without resulting in increased levels of adenosine in the CSF. Also, as suggested by animal studies [12], deoxyadenosine could mediate CNS depression even though it is a less potent CNS depressant in animal systems.

It will be important to determine the relationship between levels of adenosine or deoxyadenosine and CNS depression in more detail. The resolution of these issues is relevant in attempts to modify the scheduling of DCF to avoid this dose-limiting toxicity and when DCF is combined with ara-A, which also has neurotoxicity [14].

Acknowledgements. The authors would like to thank Dr. John MacIntyre of the Division of Biostatistics, Sidney Farber Cancer Institute and Mr. Michael Pimental, Boston University Medical Center.

This work was supported by grant no. CA 19589 from the National Cancer Institute, by a McEachern Award from the Canadian Cancer Society (PPM), and by a Junior Faculty Award from the American Cancer Society (DWK).

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Received September 26/Accepted December 23, 1980